

Luís Eduardo Almeida

**ANÁLISE HISTOLÓGICA E HISTOMORFOMÉTRICA DA REGIÃO
POSTERIOR DO DISCO DA ARTICULAÇÃO
TEMPOROMANDIBULAR HUMANA**

CURITIBA

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Dissertação apresentada ao Programa de Pós-graduação em Medicina da Pontifícia Universidade Católica do Paraná, como parte dos requisitos para obtenção do título de Mestre em Ciências da Saúde.

Orientadora: Profa. Dra. Paula Cristina Trevilatto

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DEDICO

Ao meu pai e minha mãe, Manoel e Sônia, por sempre estarem perto e terem me dado a oportunidade de adquirir o maior dos prêmios desta vida que é o estudo. Amo vocês.

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Resumo

RESUMO

Aproximadamente 3 a 4 % da população necessitam de tratamento para disfunções da articulação temporomandibular, e 70 % destes pacientes apresentam deslocamento do disco articular. O objetivo deste estudo foi analisar histológica e histomorfometricamente o disco articular da articulação temporomandibular (ATM) em grupos com e sem deslocamento do disco. Uma amostra de 39 fragmentos posteriores de disco articular (cirúrgicos = 31; grupo controle: 8) de 28 pacientes (idade média 31.2 anos) foi selecionada para estudo. Os pacientes foram considerados afetados e tratados cirurgicamente com reposição de disco quando apresentando sinais clínicos de deslocamento de disco com dor, após insucesso no tratamento não-cirúrgico por seis meses. A região posterior do disco foi removida e submetida a análises histológicas (hematoxilina-eosina) e histomorfométricas (picro-sirius, com e sem polarização). Diferenças estatisticamente significantes entre os grupos foram verificadas por meio do teste qui-quadrado ($p \leq 0,05$). O teste U de Mann-Whitney foi utilizado quando as variáveis não apresentavam distribuição normal [Teste de Kolmogorov-Smirnov(a)]. Não houve diferenças significantes entre os grupos em relação a todos os parâmetros estudados nas análises histológicas e histomorfométricas. Dentro dos limites deste estudo, não houve diferenças histológicas e histomorfométricas no disco articular da ATM entre os grupos com e sem disfunção temporomandibular.

Abstract

ABSTRACT

The aim of this study was to analyze histological and histomorphometrical features of the articular disc in groups with and without disc displacement. A convenience sample of 39 temporomandibular joints (number of case specimens: 31; number of control specimens: 8) from 28 patients (mean age 31.2) were recruited for study. The patients were considered affected and treated surgically with disc repositioning when presenting painful clinical signs of disc displacement, after unsuccessful nonsurgical treatment for at least six months. The posterior region of the disc was removed and submitted to histological (hematoxylin-eosin) and histomorphometrical (Picrosirius method) analysis. Statistically significant differences among the analyzed groups were accessed through the qui-square test ($p \leq 0.05$). The U of Mann-Whitney test was used to observe the differences among mean values when variables did not present normal distribution [Kolmogorov-Smirnov(a) test]. To the limit of this study, there were no significant structural differences in the collagenous extracellular matrix in the articular disc among groups with and without TMJ dysfunction.

Introdução

INTRODUÇÃO

Aproximadamente 3 a 4% da população necessitam de tratamento para disfunções da articulação temporomandibular (ATM) (GRAY et al., 1995) e 70 % destes pacientes apresentam deslocamento do disco articular (FARRAR & McCARTY, 1979).

Deslocamento de disco é a alteração morfológica mais comumente encontrada quando pacientes com sinais e sintomas de desordens da ATM são examinados (PASEANI et al., 1992). Frequentemente o disco está deslocado anteriormente, mas também há uma grande incidência de deslocamento medial ou lateral, ou uma combinação de ambos (BROOKS & WESTESSON, 1993; TASAKI & WESTESSON, 1993). Há dois tipos de deslocamento de disco, com e sem redução, sendo o último uma manifestação clínica mais severa (BROOKS & WESTESSON, 1993).

Nas desordens internas da articulação temporomandibular, o disco está frequentemente mal posicionado (DE BONT et al., 1987). Inicialmente acreditava-se que o deslocamento de disco precedia a degeneração do côndilo mandibular; entretanto, a alta associação de disco mal posicionado com alterações osteoartíticas da articulação tem levado muitos estudiosos a sugerir que a degeneração osteoartítica precede o deslocamento do disco (DE BONT et al., 1987).

Um dos maiores problemas na pesquisa da articulação temporomandibular em pacientes é a dificuldade na obtenção adequada de grupos controle. Isto é particularmente evidente em pacientes submetidos à cirurgia. Por razões éticas, não é possível a obtenção de biópsia de pacientes sadios, sendo o material obtido a partir de cadáveres. Vários estudos histológicos têm sido realizados nas últimas décadas (ISACSSON et al., 1986; KURITA et al., 1989; PEREIRA et al., 1996), a maioria usando espécimens de autópsia de pacientes idosos. Conseqüentemente, é difícil distinguir mudanças relativas à idade das mudanças

verdadeiramente relacionadas à degeneração da articulação. Além disso, a maioria dos estudos realizados tem focado sua área de pesquisa em características relacionadas ao processo inflamatório em si, dando pouca ou nenhuma atenção a uma caracterização mais sistemática dos vários tecidos componentes da articulação temporomandibular no seu estado sadio e no estado degenerativo. Os estudos histológicos também têm sido bastante limitados ao uso da coloração de hematoxilina e eosina (HE), e considerando outras estruturas que não o disco articular (GYNTHER et al., 1994; 1997).

Proposição

OBJETIVO

O objetivo deste estudo foi analisar histológica e histomorfometricamente a região posterior do disco articular de indivíduos com e sem disfunção da articulação temporomandibular (ATM).

Artigo

Histological and histomorphometrical analysis of posterior region of the human temporomandibular disc in individuals with disk displacement

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ABSTRACT

Purpose: The aim of this study was to analyze histological and histomorphometrical features of the articular disc in groups with and without disc displacement. **Materials and Methods:** A convenience sample of 39 temporomandibular joints (number of case specimens: 31; number of control specimens: 8) from 28 patients (mean age 31.2) were recruited for study. The patients were considered affected and treated surgically with disc repositioning when presenting painful clinical signs of disc displacement, after unsuccessful nonsurgical treatment for at least six months. Out of the control patients, 4 presented condyle fracture which needed to be opened to be reduced for treatment, and 4 displayed active condyle hyperplasia. The posterior region of the disc was removed and submitted to histological (hematoxylin-eosin) and histomorphometrical (Picrosirius method) analysis. Statistically significant differences among the analyzed groups were accessed through the qui-square test ($p < 0.05$). The U Mann-Whitney test was used to observe the differences among mean values when variables did not present normal distribution [Kolmogorov-Smirnov(a) test]. **Results:** There were no significant differences among the groups in relation to the parameters studied by histological and histomorphometrical analysis. **Conclusions:** To the limit of this study, there were no significant histological and histomorphometrical differences in the posterior region of the articular disc among groups with and without TMJ dysfunction.

Approximately 3 % to 4 % of the population seeks treatment for temporomandibular joint (TMJ) disorders,¹ and roughly 70 % of these patients suffer from disc displacement.²

Disc displacement is the morphologic alteration of the temporomandibular joint most commonly encountered when patients with signs and symptoms of temporomandibular disorders are examined.³ Most frequently the disc is displaced anteriorly, but there is also high incidence of medial or lateral displacement, as well as a combination of displacement.^{4,5} There are two types of disc displacement, with and without disc reduction, being the latter considered a more severe clinical manifestation.⁵

In internal derangements of the temporomandibular joint, the disc is often malpositioned. Initially it was believed that discal displacement precedes the onset of degeneration of the mandibular condyle; however, the high association of disc malposition with osteoarthritic changes of the joint has led many to suggest that osteoarthritic degeneration predisposes to disc displacement.⁶

One of the problems with temporomandibular joint studies in human patients is the difficulty of obtaining adequate control groups. This is particularly evident in patients when surgery is involved. For ethical reasons, it is not possible to obtain control biopsy specimens from healthy living persons, and therefore we must rely on autopsy material. Several histological studies have been performed during the past few decades⁷⁻⁹ most using autopsy specimens from older persons. Consequently, it has been difficult to distinguish age-related changes from those that occurred due to true joint disease. In a few studies, this problem has been dealt with by the use of age-matched controls^{10,11} or controls of a younger age.¹² However, most studies have focused on features related to inflammation, and little attention has been paid to a more systematic characterization of the various components in the normal state and in degenerative diseases. Moreover the histological aspects have been almost

always limited to conventional approaches, such as hematoxylin-eosin staining, and considering other anatomic structures than the articular disc.⁸

The aim of this study was to analyze histological and histomorphometrical features of the posterior region of the articular disc in groups with and without disc displacement.

MATERIALS AND METHODS

Study sample

A convenience sample of 39 temporomandibular discs from 28 patients, (mean age 31.2, range 17 to 57), were recruited for study from the patient pool of the Evangelico School Hospital, Curitiba, PR, Brazil, approved by the Ethical Committee in Research at Pontifical Catholic University of Paraná (PUCPR), according to the Resolution 196/96 of the National Health Council, approved under registration number 104. Subjects did not have any of the following exclusion criteria: use of orthodontic appliances; chronic usage of anti-inflammatory drugs; a history of diabetes, hepatitis or HIV infection; immunosuppressive chemotherapy; history of any disease known to severely compromise immune function; current pregnancy or lactation; dentofacial deformities; previous TMJ surgery, and previous steroid injection in the TMJ.

Subjects completed personal medical history questionnaires, and within a protocol approved by an Institutional Review Board, signed a consent form after being advised of the nature of the study. All patients were asked to complete a pain questionnaire, and a clinical examination was performed by the operating oral and maxillofacial surgeon.

The patients were considered affected and treated surgically when presenting painful clinical signs of disc displacement, after unsuccessful nonsurgical treatment for at least six months.

Subjects were included in clinical categories according to the presence or absence of disc displacement:

Group 1: patients presenting anterior disc displacement with reduction (ADDWR) (n=15; 23 specimens);

Group 2: patients presenting anterior disc displacement without reduction (ADDW/OUT) (n=5; 8 specimens);

Group 3: subjects without any signs of disc displacement (control group; n=8; 8 specimens).

In the control group, four individuals presented condyle fracture (CFx) which needed to be opened to be reduced for treatment, and four displayed active condyle hyperplasia (CH).

Table 1 shows the baseline characteristics of the groups.

Surgical technique

General anesthesia was administered via nasotracheal intubation in all patients. An endaural incision was done to gain access to the TMJ area. The superior joint space was entered and the capsular attachments were incised anteriorly beyond the articular eminence and posteriorly along the posterior wall of the fossa. The anterior, lateral, and the medial ligamentous attachments were freed if needed, to permit passive repositioning of the disc over the condylar head. The excessive bilaminar tissue was resected and a small portion of the disc posterior band was removed. At this step a modification in Mehra and Wolford's technique¹³ was developed, in which, instead of using a Mitek anchor, a regular 2.0 mm titanium screw was used. A 2 x 8 mm hole was made in the posterior head of the condyle with a standard drill bit (1.6 mm diameter). The screw was positioned 8–10 mm below the superior aspect of the condyle. Prior to placing the screw, one size 2.0 Ethibond (Ethicon, Inc., Somerville, NJ, USA), braided polyester, permanent suture was tied around the head of the

screw. The screw was then placed into the prepared hole. The sutures are tugged to ensure proper seating and stability of the screw. The two Ethibond sutures were then attached to the disc in a mattress fashion. The capsule, subcutaneous tissue, and skin were then closed in a routine manner. Postsurgical physical therapy was indicated at the discretion of the surgeon.

Histological and histomorphometrical analysis

The specimens were obtained from the posterior segment of the disc. They were fixed by immersion in 10 % neutral-buffered formaldehyde solution. Subsequently they were embedded in paraffin wax, and 4 µm thin sections were stained with hematoxylin and eosin (HE) or by the Picrosirius method, which consists in staining sections for 1 h in an 0.1% solution of Sirius Red (Sirius Red F3B200, Mobay Chemical Corp., USA) in saturated picric acid and analysis by polarizing microscopy. The tissue sections were examined on a American Optical BX50 microscope and the morphometrical analysis was performed with the Image *Proplus*® software (Media Cybernetics, Inc. Silver Spring, MD. USA).

Histologic analysis

One coded hematoxylin-eosin slide (the most representative of 5) per sample was analyzed without knowledge of group identification (blinded). The following parameters were analyzed: degree of vascularization (absence, mild and moderate/severe), presence of blood vessels at the disc periphery, hyperemia, synovial hyperplasia, synovial vilosity, presence of chondrocytes/fibrocytes, hyalinization/fibrosis (absence, mild and moderate/severe), inflammatory infiltrate, presence of adipocytes. All parameters of interest were estimated three times, and the mean value was then calculated.

Histomorphometrical analysis

Sirius Red stained tissue sections were analyzed without knowledge of group identification (blind). The slides were examined i) without polarized light, which gives the total amount of

collagen, and ii) with polarized light, using a binocular microscope attached in a computerized image analysis system (*Image Proplus*® software). The image software was calibrated in micrometers for a 20 x objective and the density of the tissue sample was calibrated by the range 0 to 2, where: i) 0 = total light getting through the tissue (low density), and ii) 2 = no light getting through the tissue (high density). After the calibration, specific areas of Sirius Red staining slides (magenta) were captured (without polarized light). The software performed the sum of mean density in each area and the sum of the total area by color histogram function.

The same procedure was performed with Sirius Red stained sections using polarized light, and the software was set up to distinguish between the two interference colors that could be observed on the tissue sections, yellow or red. 17

Statistical Analysis

Statistical significant differences among the analyzed groups were accessed through the chi-square test (χ^2) at the level of probability $p < 0.05$. When expected frequencies were smaller than 5, in 2X2 tables, the Fisher test was also used. The U Mann-Whitney test was used to observe the differences among the mean values for the variables: degree of vascularization, hyalinization/fibrosis, and collagen area and density for the groups, once these variables did not present normal distribution in relation to the groups by the Kolmogorov-Smirnov(a) test.

RESULTS

There were no significant differences among the groups in relation to all the parameters studied by HE. Regarding histometrical analysis, no differences among the groups were observed for Sirius Red staining, either using or not polarized light. No differences were also noted when groups 1 (ADDWR) and 2 (ADDW/OUT) were considered together versus the control group (group 3). Vascularization was associated with inflammatory infiltrate ($p=0.029$).

Figure 1 shows common histological aspects of a control and an affected posterior region articular disc, and figure 2 presents aspects of control and diseased discs under polarization.

DISCUSSION

A few histological studies have been performed on the human TMJ disc in disease.⁷⁻⁹ However, the interpretation of these has been hampered by the lack of healthy controls¹³⁻¹⁵ or the analysis of healthy control specimens only from old individuals⁷⁻⁹ In this study individuals without disc displacement of any kind and no clinical signs of temporomandibular joint dysfunction were considered belonging to the control group. Although individuals in the control group were submitted to mandibular trauma or presented condyle hyperplasia, their discs lacked degenerative alterations caused by aging. This avoids that findings observed in asymptomatic joints may be attributed to aging rather than pain.⁹ Cadaveric specimens, even if the sample is collected immediately after death, are generally from old and systemically impaired patients.

Regarding sex, Paegle et al.¹⁶ observed no significant differences between female and male joint specimens on morphological structures. Thus, it appears to be unnecessary to match controls by gender.

A strong aspect of this study is that disc itself was considered for study. Most studies have been conducted not right in the disc but rather in adjacent tissues such as retrodiscal and posterior attachments.¹⁴⁻¹⁶

This study compared histological and histomorphometrical features of painful and asymptomatic TMJ discs. No differences among the groups were observed concerning degree of vascularization, presence of blood vessels at the disc periphery, hyperemia, synovial hyperplasia, synovial vilosity, presence of condrocytes/fibrocroncytes, hyalinization/fibrosis,

inflammatory infiltrate, and presence of adipocytes. An inflammatory cellular infiltrate was seldom observed in our material, which is in accordance with several other earlier studies^{7,8,12} and associated with degree of vascularization. No differences were also observed in the collagenous extracellular matrix. The birefringence brightness and interference colors are directly related to the optical retardations. The yellow and red interference colors observed in the collagenous extracellular matrix represent, therefore, distinct compactness of collagen fibrils in the same tissue section, where the red color represents a higher optical retardation¹⁸. The lack of histological differences among the study groups may be attributed to the fact that the disc is a continually remodeling anatomic structure which is in a progressive process of healing in response to trauma. Further studies should be carried out, especially regarding a greater number of samples, to corroborate these findings.

In conclusion, there were no significant histological and histomorphometrical differences in the posterior region of the articular disc among the groups with and without TMJ dysfunction.

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Table 1. Baseline clinical characteristics of the study group with and without TMJ disfunction.

Patient (N=28)	Race	Sex	Age (years)	Diagnosis	Affected Side (Right)	Affected Side (Left)
1	Caucasian	Female	35	ADDW/OUTR	X	X
2	Caucasian	Female	24	ADDW/OUTR		X
3	Caucasian	Female	23	ADDW/OUTR	X	X
4	Caucasian	Female	45	ADDW/OUTR	X	
5	Caucasian	Female	32	ADDW/OUTR	X	X
6	Caucasian	Female	30	ADDWR		X
7	Afro-American	Female	20	ADDWR		X
8	Afro-American	Female	57	ADDWR		X
9	Caucasian	Female	33	ADDWR	X	
10	Caucasian	Female	25	ADDWR	X	X
11	Caucasian	Female	22	ADDWR	X	X
12	Caucasian	Female	42	ADDWR	X	
13	Caucasian	Female	56	ADDWR	X	
14	Caucasian	Female	26	ADDWR	X	X
15	Caucasian	Female	38	ADDWR	X	X
16	Caucasian	Female	37	ADDWR	X	X
17	Caucasian	Female	36	ADDWR	X	X
18	Caucasian	Female	34	ADDWR		X
19	Caucasian	Female	46	ADDWR	X	X
20	Caucasian	Female	26	ADDWR	X	X
21	Caucasian	Female	17	CH		X
22	Caucasian	Female	43	CH		X
23	Caucasian	Female	40	CH		X
24	Caucasian	Female	18	CH		X
25	Caucasian	Male	26	C FX	X	
26	Caucasian	Female	30	C FX		X
27	Caucasian	Male	27	C FX	X	
28	Caucasian	Male	18	C FX		X

ADDW/OUTR = Anterior disc displacement without reduction

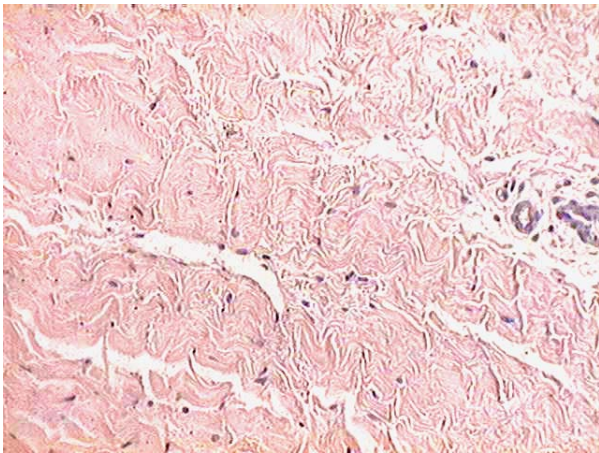
ADDWR = Anterior disc displacement with reduction

CH = Condylar hyperplasia

C FX = Condylar fracture

Figure 1. Histologic aspects (HE) of articular discs from a control (A) and an affected (B) individual.

A.



B.

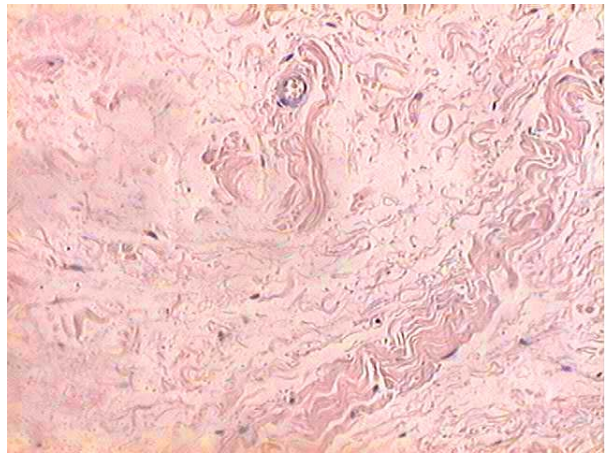
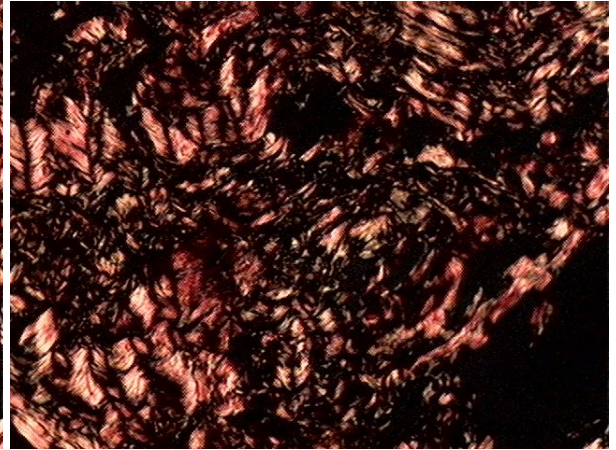


Figure 2. Aspects of the articular disc of control (A) and diseased (B) patients (picro-Sirius Red under polarization).

A.



B.



Conclusão

CONCLUSÃO

Dentro dos limites deste estudo, não houve diferenças histológicas e histomorfométricas na região posterior do disco articular da ATM entre os grupos com e sem disfunção temporomandibular.

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